The current DNT-in vitro battery (DNT-IVB) for regulatory application

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INTRODUCTION

Testing for developmental neurotoxicity (DNT) is currently performed using rats according to the OECD/US-EPA guidelines. It is broadly accepted that in vitro methods allow a more efficient toxicity testing for hazard identification than traditional animal experiments, concerning cost, time and extrapolation of testing results to humans. Due to these obstacles, a DNT in vitro testing battery (DNT-IVB) has been assembled under the guidance of the European Food Safety Authority (EFSA) in collaboration with the Danish- and US-Environmental Protection Agency that was challenged with 119 chemicals (Masjosthusmann et al. 2020). Here, results of the DNT-IVB will be presented.

METHODOLOGY

The test methods are based on primary human neural progenitor cells (hNPC), human induced pluripotent stem cell (hiPSC)-derived neural crest cells and neurons, as well as LUHMES cells and model the following key neurodevelopmental processes, i.e. hNPC proliferation, migration and differentiation into neurons and oligodendrocytes, neurite morphology (NPC1-5), neural crest cell migration (UKN2), neurite outgrowth of central (UKN4) and peripheral (UKN5) neurons. In addition, a hiPSC-based test method for neuronal network formation and function (hNNF assay) was set up and challenged with 28 pesticides.

RESULTS

Concentration-response curves reveal benchmark concentrations (BMCs) for the 119 compounds in the individual test methods. Classification models for data interpretation were applied. For interpretation of compound results across the whole battery, respective of most sensitive endpoints (MSEs) were determined. Battery results were used in two case studies, i.e. hazard characterisation of deltamethrin and flufenacet in an Adverse Outcome Pathway-informed Integrated Approach for Testing and Assessment (by EFSA) and flame retardant prioritisation (by the US-National Toxicology Program).

DISCUSSION

These data demonstrate the successful set-up of a DNT-IVB that can be used for different regulatory purposes. More assays complementing the current neurodevelopmental key events are underway for increasing confidence in the battery. An OECD (Organisation for economic collaboration and development) guidance document is currently being prepared that informs on use and interpretation of the DNT-IVB for regulatory application.